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Original Research Article

Evaluation of protective potency of coconut oil-based probiotic against antibiotic-induced compromised gastrointestinal attributes and associated complications in Swiss albino mice

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ABSTRACT

Background: Antibiotic-induced gastrointestinal toxicity is a major concern globally. The gut-organ axis has been explicitly studied, and hence, any damage to the gut ecosystem directly or indirectly manifests into compromised multi-organ functions. Therefore, the present study was designed to explore the protective potency of coconut oil-probiotic (C-PRO) against antibiotic-induced compromised gastrointestinal attributes and associated complications.
Methods: Animals were divided into 5 groups (n=6). Normal saline was given to the control group; the antibiotic cocktail was given to the antibiotic group. In the treatment group, low and high doses of C-PRO were given post-antibiotic treatment. In the per se group, only a high dose of C-PRO was given. After 28 days, animals were studied for neurobehavioral parameters and scarified. Blood and organs were collected and stored for histopathological, immune histochemical, and biochemical analysis.
Results: Antibiotic treatment reduced body weight, increased oxidative stress, and caused histopathological damage to the stomach, duodenum, colon, brain, heart, liver, lungs, kidney, spleen, and testis. It also altered biochemical parameters such as lactate dehydrogenase (LDH), creatine kinase-myocardial band (CK-MB), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), albumin, creatinine, urea, uric acid, and blood urea nitrogen (BUN). Additionally, Antibiotic exposure in mice exhibited depressive-like behaviour and declined cognitive function. The treatment with a low dose of C-PROs showed negligible protective effect, whereas a high dose of C-PRO effectively reversed the structural, biochemical, and neurobehavioral attributes toward normal.
Conclusions: We conclude that the synergistic effect of coconut oil and probiotics, which have potent antioxidant and anti-inflammatory effects, might be responsible for multidimensional protective effects against compromised gastrointestinal attributes and associated complications.

Keywords: Coconut oil, Probiotics, Dysbiosis, Inflammation, Oxidative stress, Neurobehavioral, Gut-organ axis

INTRODUCTION

Antibiotics are included in essential medicines due to their ability to fight a broad spectrum of infectious diseases caused by microbes and, hence, become an important part of treatment. Conversely, the excess or off-label use of antibiotics resulted in numerous clinical complications like diarrhea, gut microbiota metabolic disorder, superinfection, and antibiotic resistance.[1,2] Among all, specifically, broad-spectrum antibiotics like metronidazole, ampicillin, clindamycin, and vancomycin are reported to alter the overall balance of bacterial

composition and lead to multiple complications, as reported in *in vivo* models.[2,3] Subsequently, a large intake of antibiotics promotes the release of pro-inflammatory cytokines along with intestinal mucosal damage. It is observed that any alteration in pro-inflammatory cytokines and intestinal mucosal was directly associated with multiorgan toxicity that is manifested biochemically and histopathologically.[2]

It is also well established that the gut flora includes a complex fusion of different microbes that facilitates various physiological processes in the host. It facilitates gut-brain communications, gut-liver axis, and various other multiorgan complications.[4] The clinical research proves the correlation of intestinal dysbacteriosis with diverse pathological conditions of the nervous system, which results in symptoms of inflammatory conditions and irritable bowel syndrome (IBS).[4]The research data applying the hot plate test suggests that the treatment of young, healthy rodents with antibiotics incites depressive behaviors without altering pain reactions.[5] In mouse models, oral consumption of antibiotics diminishes mechanical allodynia and thermal hyperalgesia in chronic constriction injury of the sciatic nerve.[6] Through various epidemiological studies, it is anticipated that the pain reaction declines in animals with intestinal dysbacteriosis. Moreover, research also suggests changes in spontaneous pain and experiential emotions in IBS patients and IBS mice.[7,8] Above and beyond, it has been seen that treatment with probiotics helps in reducing brain ailments caused by altered microbiota.[9] Though probiotics are considered a possible treatment for antibiotic-induced dysbacteriosis. However, their effectiveness and primary mechanisms are yet to be answered. Therefore, more research should be conducted to point out the exact mechanism of action of probiotics in antibiotic-induced multiorgan toxicities.

In the recent findings, it has been well established that probiotics not only avert the dysbiosis of the gut microbiota due to antibiotic use but also attack opportunistic pathogens. Hence, probiotics might have additional benefits when used alongside antibiotics. In common treatment methods, where a combination of antibiotics and C-PROs was used, the outcomes were favorable and considered as a potential approach for the treatment of SIBO.[10] Theoretically, the combination treatment of antibiotics and C-PROs should eliminate existing pathogenic bacteria of the small intestine, clean toxins, and rebuild the defending gut microbiota, which will eventually manifest into multi-organ protection. It is also well-studied that Coconut (*Cocos nucifera*) oil consists of mono laurin comprised of short-chain fatty acid (lauric acid),which is an antimicrobial and anti-inflammatory monoglyceride has the potential to disrupt themembrane structure of microbes.[11] The notableantimicrobial activity of coconut oil on the skin has confirmed its ability to prevent the division of known skin pathogens like *Propioni bacterium acnes* and *Staphylococcus aureus.*[12] A different study exhibits an increase in good bacteria like lactobacillus, *Allobaculum*, and *Bifido bacterium* species in the intestines using coconut oil.[13] Studies using different animal models have revealed low antimicrobial activity of lauric acid against lactic acid bacteria in the human gut but high activity against *E. coli* and *Clostridium.*[13] Various other studies have also shown coconut oil's potent antioxidant, anti-inflammatory, nephroprotective, cardioprotective, hepatoprotective, neuroprotective, and anticancer properties.

Considering these facts, in the present study, we have explored the combination of coconut oil and probiotics for their protective effect and associated multiorgan complications. Hence, we have estimated the various biochemical parameters associated with multi-organs along with immune histochemical and neurobehavioral studies.

METHODS

For the induction of gut toxicity antibiotics, ampicillin (100 mg/kg), neomycin sulfate (100 mg/kg), metronidazole (100 mg/kg), and vancomycin (50 mg/kg) were procured from Alkem Pharmaceuticals Pvt. Limited. C-PRO used in the experiment was obtained from Herbalfarm Lifecare Private Limited, New Delhi, as a gift sample. All other chemicals and reagents used in the experiment were of analytical grade.

Experimental animals

Swiss albino mice (n=30; 22.5±3 g) were obtained from the central animal house facility of the Institute for Innovative Research, Patna, after approval from the Committee for the Purpose of Control and Supervision of Experiments on Animals (01/IAEC/IIR/2024).

All animals had unimpeded access to food and drinking water and were kept in a controlled room (temperature, 25±2 °C; relative humidity, 45–60%; lighting cycle, 12 h/d; 06:00–18:00 for light) for the first 7-day acclimation period.

Animals' experimental design

Mice were given 1 week (week 1) to acclimatize to experimental housing conditions (temperature maintained at 23±1°C, humidity fixed at 55±10%, 12/12-h light/dark cycle). Afterwards, they were divided into five groups (n=6). Group I; control (normal saline for 28 days), group II; antibiotic cocktail [through oral gavage (100 µl/mouse], daily for 28 days), group III; Antib [through oral gavage (100 µl/mouse] + low dose C-PRO (0.5 ml/kg, orally for 28 days), group IV; Antib [through oral gavage (100 µl/mouse] + high dose C-PRO (1 ml/kg orally), and group V; drug per se (1 ml/kg C-PRO, orally for 28 days). On day 29, mice were tested for neurobehavioral parameters and euthanized.[2,3] Serum and multiorgan were collected from all animals and kept at −80 °C for further experiments.

Estimation of the marker of oxidative stress

Markers of oxidative stress such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and thiobarbituric acid reactive substances (TBARS) were done using spectrometric analysis, and as per the previously published methods of Marklund and Marklund, Claiborne and Fridovich, Sedlak and Lindsay and Ohkawa et al.[14-17]

Biochemical estimation

Markers of liver injury (ALT, AST, GGT, and albumin), kidney injury markers (creatinine, urea, uric acid, and blood urea nitrogen), cardiac injury markers (LDH and CK-MB), lipid profile (cholesterol, triglyceride, LDH, and HDL) and hematological parameters (Hb, RBC, WBCs, and platelets) were estimated using an autoanalyzer.

Hematoxylin-Eosin staining

Once the animals were sacrificed, organs such as the stomach, small intestine, large intestine, heart, liver, kidney, lung, spleen, testis, and brain were removed and kept in 10% formalin solution and embedded in paraffin for hematoxylin and eosin (HE) staining. The stained slides were examined under a light microscope.[3]

Forced swimming test

The animals were forced to swim individually in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 degrees Celsius. After an initial period of vigorous activity, each animal assumed a typical immobile posture. They were forced to swim for 6 minutes, and their immobility was recorded.[18]

Step-down latency test

A step-down latency (SDL) test was performed to analyze the cognitive function. In this process, animals were first given a 5-minute training session in which a chamber with a wooden grid was placed in the center. The base or floor of the chamber comprises an electric grid. In the training session, animals were kept in a wooden box, and as they stepped down, an electric shock was applied. As a result, the animals moved back to the wooden box. In the test session, when the animals were again placed on a wooden box, the time taken to step down on the electric grid was recorded. Increased timing to step down signifies a stronger memory.[19]

Statistical analysis

All data are articulated as the mean±standard deviations (SD). Collation data was made among multiple groups via one-way analysis of variance (ANOVA) test. P<0.05 was regarded as pointing out a statistically substantial difference.

RESULTS

Effect of C-PROs on Antib-induced derailed body weight

In the present study, we found a significant reduction in body weight from week 1 to week 4 ($p<0.05$ for week 1 and $p<0.001$ for weeks 2, 3, and 4, respectively) when compared to the control group. When the change in BW was studied for low and high doses of C-PROs, we found that low doses failed to exhibit any significant increment in body weight from week 1 to week 4 ($p>0.05$). When the change in body weight was studied for the high dose of C-PRO, we found no significant increase in BW after one week ($p>0.05$) of treatment, whereas a significant increment was seen from week 2 onwards ($p<0.001$). The drug per se group had almost similar effects, as exhibited by the control group.

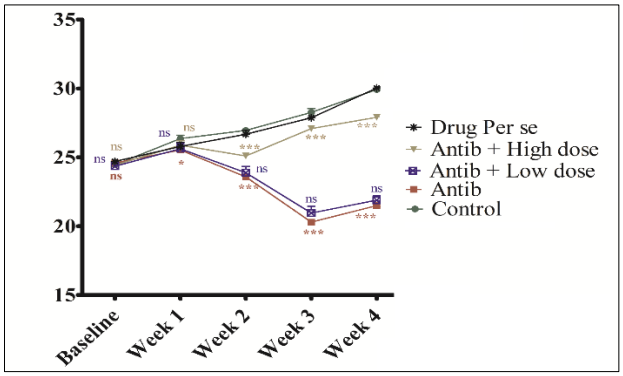

Figure 1: The effect of low and high doses of C-PRO on derailed body weight. One-way ANOVA and Tukey's multiple comparison tests were employed for statistical analysis.

Effect of C-PRO on Antib-induced oxidative stress

In the present study, we found the reduced antioxidant activity of SOD, CAT, and levels of GSH, along with a reduced level of TBARS, in the Antib-treated group when compared to control groups ($p<0.001$). When comparing the antioxidant effects of low-dose and high-dose C-PROs, we found that low doses of C-PROs showed no significant effect against reduced SOD ($p>0.05$), CAT ($p>0.05$), and GSH ($p>0.05$), but showed a mild antioxidant effect against an increased level of TBARS ($p<0.05$). When the antioxidant effect of a high dose of C-PRO was compared against Antib, we found the significantly increased antioxidant activity of SOD ($p<0.001$), CAT ($p<0.01$), increased level of GSH ($p<0.001$), and significantly reduced level of TBARS ($p<0.001$). The drug per se group had almost similar effects, as exhibited by the control group.

Effect of C-PRO on Antib-induced histopathological damage in stomach and duodenum

In the present study, we found significantly damaged stomach and duodenum in the Antib-treated group when

compared to the control group. Marked cellular disintegration, inflammatory changes, mucosal damage, and damaged glands were seen in the antibody-treated group. The low-dose treated group did not show any marked improvement in the damaged structural attributes of the stomach and duodenal section. However, a high dose of C-PROs showed marked improvement and effectively reversed the damaged attributes toward normal. The drug perse group had almost similar effects, as exhibited by the control group.

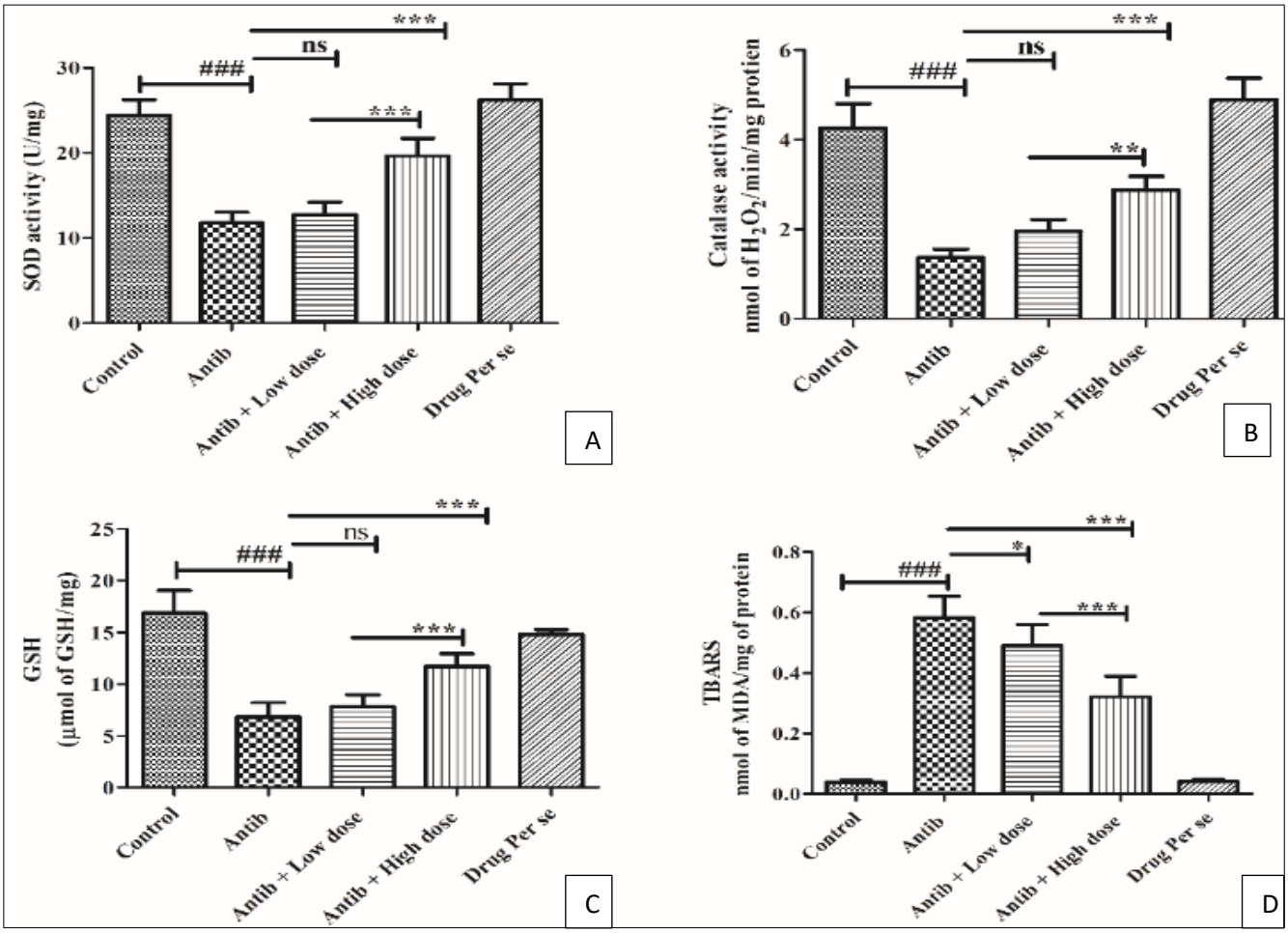

Figure 2 (A-D): The antioxidant effect of low doses and high doses of C-PRO against their derailed activity and level. One-way ANOVA and Tukey's multiple comparison tests were employed for statistical analysis.

Effect of C-PRO on Antib-induced histopathological damage in colon

In the present study, we found a significantly damaged colon and reduced length of colon ($p<0.001$) in the Antib-treated group when compared to the control group. Marked cellular disintegration, inflammatory changes, mucosal damage, and damaged glands were seen in the antibody-treated group. The low-dose treated group did not show any marked improvement in the damaged structural attributes, and no improvement was seen against the reduced colon length ($p>0.05$) of the colon section. However, a high dose of C-PROs showed marked improvement, and effectively reversed the damaged attributes towards normal, and also increased the length of the colon ($p<0.001$) considerably when compared to the Antib-treated group. The drug per se group had almost similar effects, as exhibited by the control group.

Effect of C-PRO on Antib-induced histopathological damage in colon

As seen in Figure 5, the upper panel represents the histopathology of the testis, the middle panel is the splenic section, and the lower panel represents the lung section. The control group from the testis, spleen, and lung represents healthy structural attributes that can be seen in terms of intact seminiferous tubules, Leydig cells, Sertoli cells, and spermatogonia (testis), distinguished red pulp, white pulp, marginal zone, and hematopoietic cells (spleen), no congestion, inflammatory infiltrates, or thickening of the alveolar wall (lung). In the Antib-treated group, marked damage to testicular tissue, spleen, and lung

was seen and manifested in terms of damaged seminiferous tubules, cellular disintegration and congestion (testis), distorted and undistinguishable red-white pulp, and marginal zone along with reduced hematopoietic tissue (spleen), severe congestion, macrophagic infiltration and thickening of the alveolar wall in the lung tissue was seen. The lower dose of C-PRO did not show any significant effect in reversing the histopathological damage whereas a high dose of C-PRO effectively reversed the structural damage towards normal.

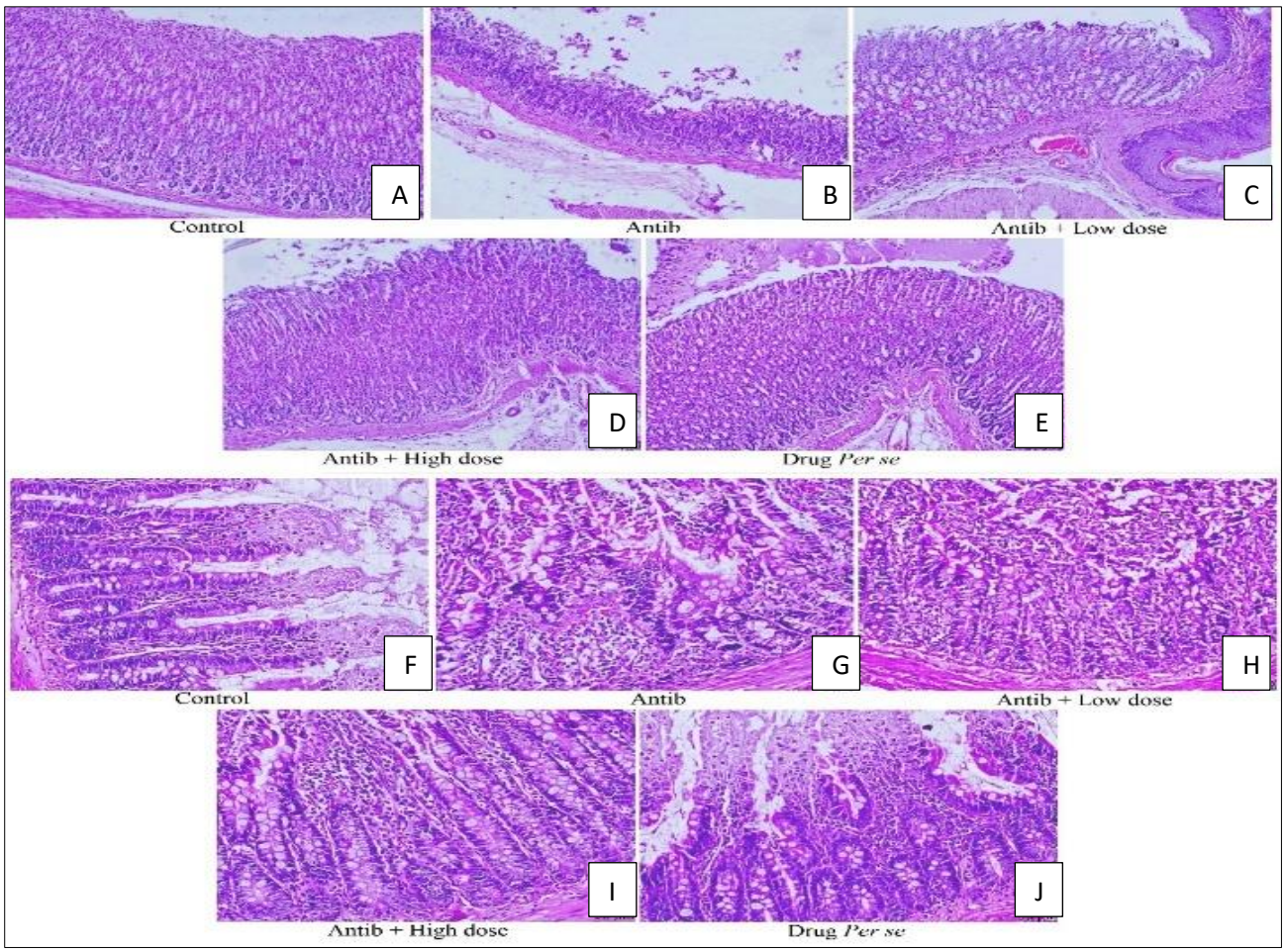

Figure 3 (A-J): Histopathological damage in the Antib-treated group in the stomach and duodenal tissue. The low dose of C-PRO failed to exhibit reversal of damage, whereas high dose of C-PRO effectively reversed the damaged stomach and duodenal section towards normal. H&E; 50 µm; 200x.

Effect of C-PRO on Antib-induced cardiac damage

In the present study, the control group showed normal histopathology of cardiac tissue, with no cellular disintegration, congestion, pyknosis, apoptosis, inflammatory infiltrates, or myocardial disintegration was found. Also, serum levels of LDH and CK-MB were found to be minimal. On the contrary, in the Antib-treated group, we found marked myocardial disintegration, pyknosis, and widening of myofibrils when compared to the control group ($p<0.001$). Also, in the Antib-treated group, the serum levels of LDH and CK-MB were elevated ($p<0.001$) when compared to the control group. The low-dose treated group did not show any marked improvement in the damaged structural attributes and against elevated levels of LDH but significantly reduced the level of CK-KM ($p<0.05$). However, a high dose of C-PROs showed marked improvement, effectively reversed the structurally damaged attributes towards normal, and also reduced the levels of LDH ($p<0.001$) and CK-MB ($p<0.001$) considerably when compared to the Antib-treated group. The drug perse group had almost similar effects, as exhibited by the control group.

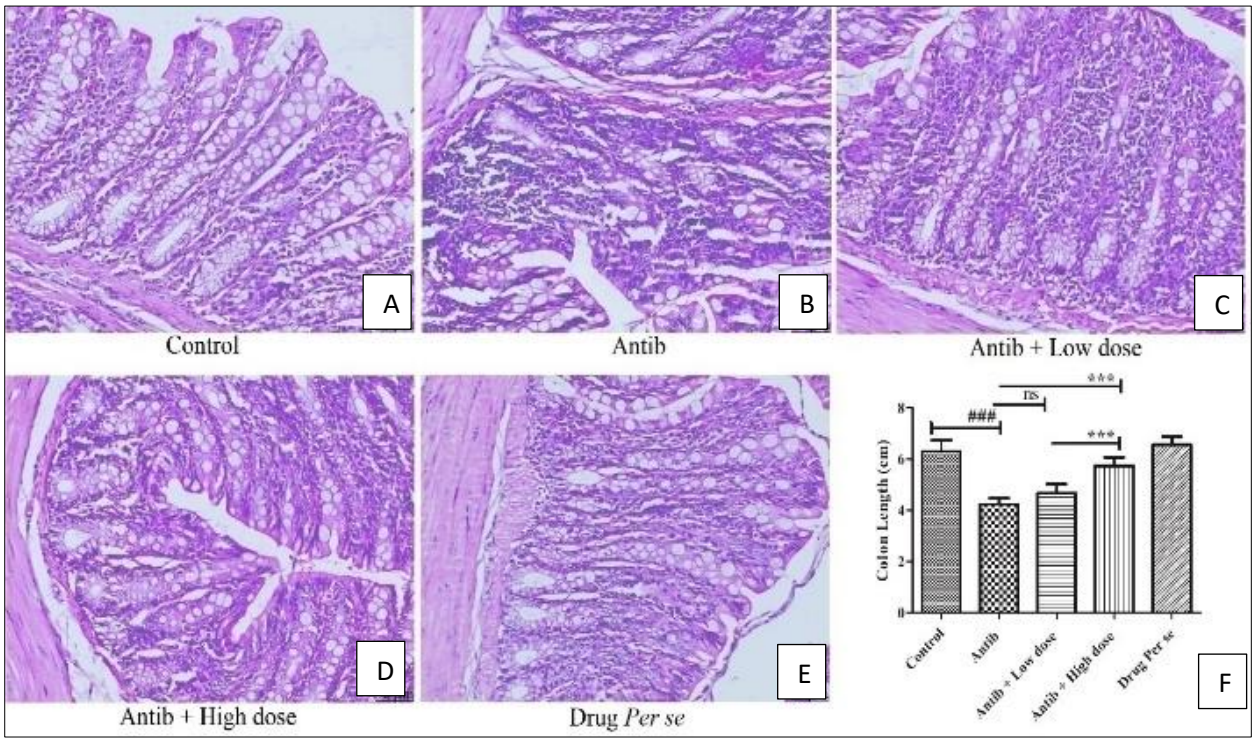

Figure 4 (A-F): Histopathological damage and compromised length of colon in the Antib-treated group. The low dose of C-PRO failed to exhibit reversal of damage, whereas the high dose of C-PRO effectively reversed the damaged colon section towards normal. H&E; 50 µm; 200x.

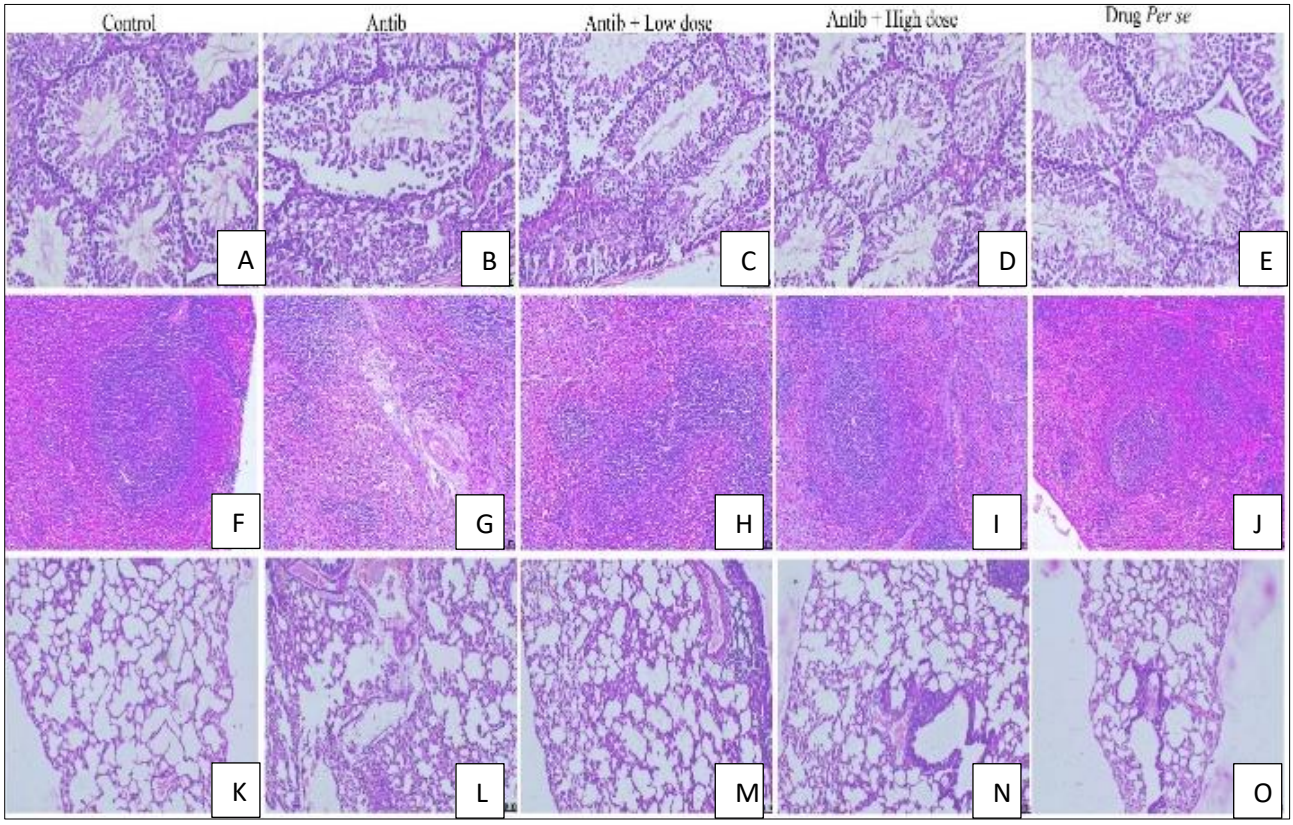

Figure 5 (A-O): The upper, middle, and lower panels show the histopathological analysis of the testis (400x), spleen (200x), and lung (100x). As seen, the low dose of C-PRO failed to exhibit reversal of damage, whereas the high dose of C-PRO effectively reversed the damaged sections towards normal.

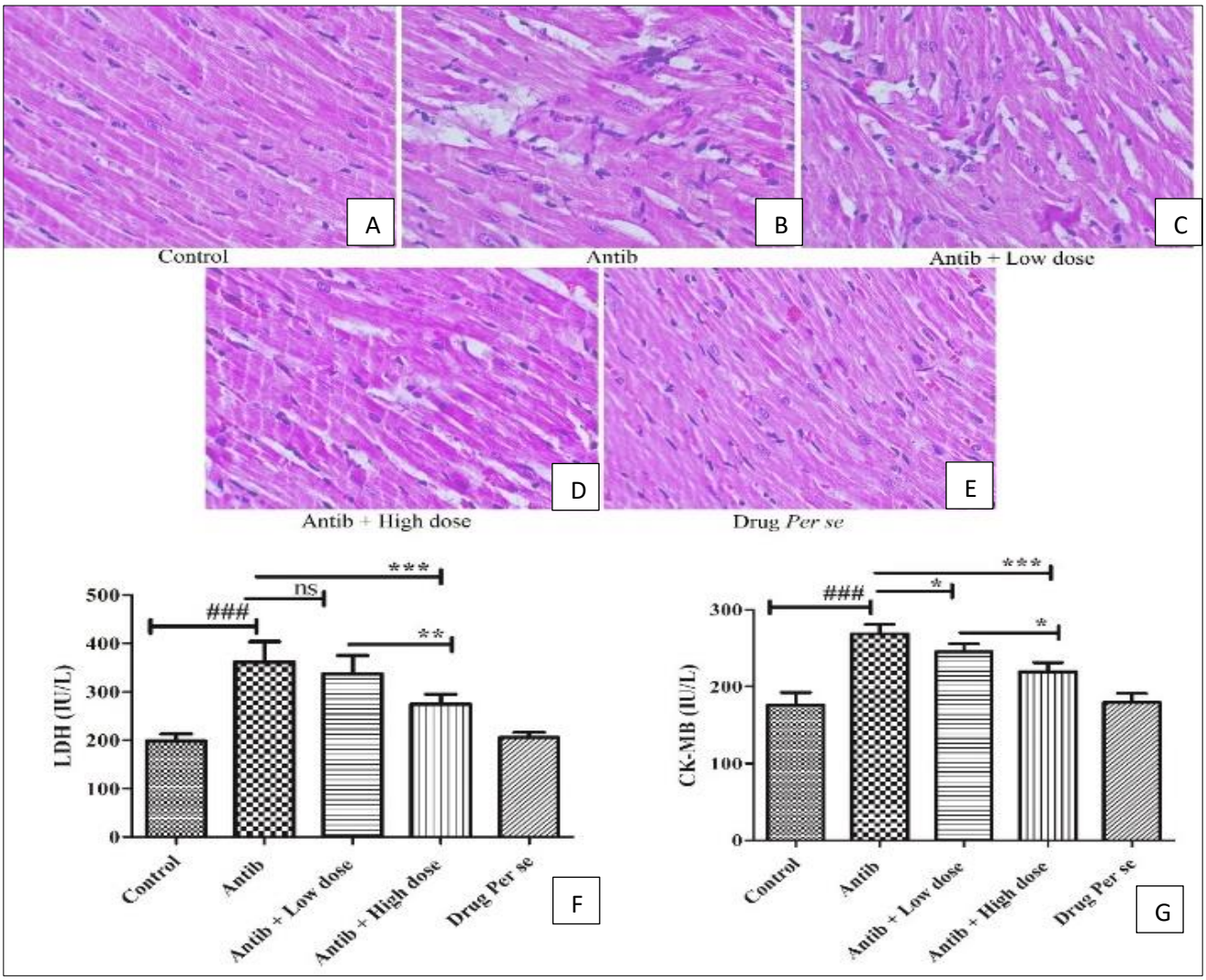

Figure 6 (A-G): Histopathological damage and compromised serum markers of cardiac damage in the Antib-treated group. The low dose of C-PRO failed to exhibit reversal of structural damage and biochemical parameters, whereas the high dose of C-PRO effectively reversed the structural damage and biochemical parameters towards normal. H&E; 50 µm; 400x.

Effect of C-PRO on Antib-induced hepatic damage

In the present study, the control group showed normal histopathology of hepatic tissue where no cellular disintegration, congestion in the central vein, pyknosis, apoptosis, inflammatory infiltrates, or hepatic fibrosis was found. Hepatocytes appeared normal and were polygonal in shape. Also, serum levels of ALT, AST, GGT, and albumin were found to be minimal. On the contrary, in the Antib-treated group, we found marked hepatocytic disintegration, pyknosis, fibrotic changes, and congestion in the central vein when compared to the control group ($p<0.001$). Also, in the Antib-treated group serum levels of ALT, AST, GGT, and albumin were elevated ($p<0.001$) when compared to the control group. The low-dose treated group did not show any marked improvement in the damaged structural attributes of liver tissue and against elevated levels of serum levels of ALT, AST, and GGT ($p>0.05$), but significantly reduced the level of albumin ($p<0.05$). High dose of C-PROs showed marked improvement, effectively reversed the structurally damaged attributes towards normal, and reduced the level of ALT, AST, GGT, and albumin ($p<0.001$) considerably when compared to the Antib-treated group. The drug perse group had almost similar effects, as exhibited by the control group.

Effect of C-PRO on Antib-induced renal damage

In the present study, the control group showed normal histopathology of renal tissue where no cellular disintegration, congestion, pyknosis, apoptosis, inflammatory infiltrates, or renal fibrosis was found. Glomerulus, Bowman's capsule, basement membrane, PCT, and DCT appear normal. Also, serum levels of creatinine, uric acid, BUN, and urea were found to be minimal. On the contrary, in the Antib-treated group, we found disintegration of PCT, DCT, and glomerulus along

with damaged basement membrane and marked pyknosis. Additionally, serum levels of creatinine, uric acid, BUN, and urea were significantly elevated when compared to the control group ($p<0.001$). The low-dose treated group did not show any marked improvement in the damaged structural attributes of renal tissue and against elevated levels of serum level of creatinine, uric acid, and BUN ($p>0.05$) but significantly reduced the level of urea ($p<0.05$). High doses of C-PROs showed marked improvement and effectively reversed the structurally damaged attributes towards normal and reduced the level of creatinine, uric acid, BUN, and urea ($p<0.001$) considerably when compared to the Antib-treated group. The drug perse group had almost similar effects, as exhibited by the control group.

Effect of C-PRO on Antib-induced neuronal damage

In the present study, the control group showed normal histopathology of brain tissue (hippocampus and cortex), where no neuronal disintegration, pyknosis, or inflammatory infiltrates were found. Considering the neurobehavioral outcome using the FST and SDL tests, minimal duration of immobility (FST) and increased duration of retention transfer latency. On the contrary, in the Antib-treated group, we found an elevated percentage of degenerated neurons in the hippocampus ($p<0.001$) and cortex ($p<0.001$), increased duration of immobility ($p<0.001$), and reduced duration of retention transfer latency ($p<0.001$) when compared to the control group. The low-dose treated group did not show any marked improvement in the damaged structural attributes of the hippocampus and cortex ($p<0.05$). Also, treatment with the low-dose C-PRO showed mild improvement in the neurobehavioral attribute in terms of FST, where the durat ion of immobility was significantly reduced ($p<0.05$), but a marked improvement was seen for the SDL ($p>0.05$). High doses of C-PROs showed marked improvement and effectively reversed the structurally damaged attributes of the hippocampus and cortex toward normal ($p<0.001$). High doses of C-PROs also reduced the duration of immobility ($p<0.001$) and increased the duration of retention transfer latency when compared to the Antib-treated group. The drug per se group had almost similar effects, as exhibited by the control group.

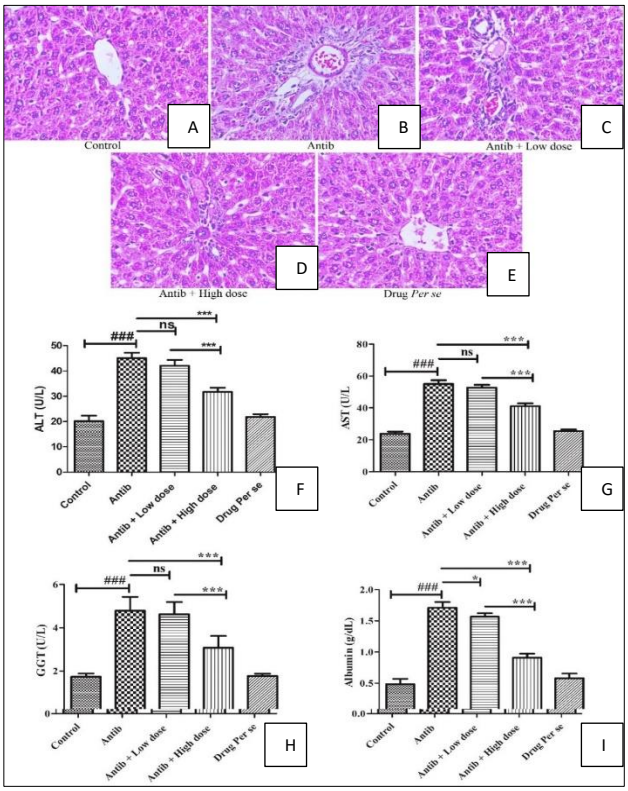

Figure 7 (A-I): Histopathological damage and compromised serum markers of hepatic damage in the Antib-treated group. The low dose of C-PRO failed to exhibit reversal of structural damage and biochemical parameters, whereas the high dose of C-PRO effectively reversed the structural damage and biochemical parameters towards normal. H&E; 50 µm; 400x.

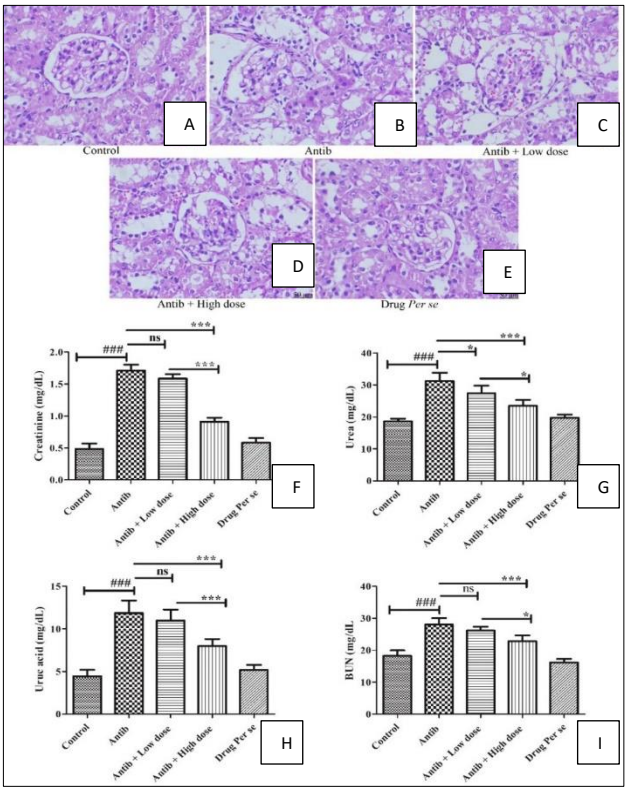

Figure 8 (A-I): Histopathological damage and compromised serum markers of renal damage in the Antib-treated group. The low dose of C-PRO failed to exhibit reversal of structural damage and biochemical parameters, whereas the high dose of C-PRO effectively reversed the structural damage and biochemical parameters towards normal. H&E; 50 µm; 400x.

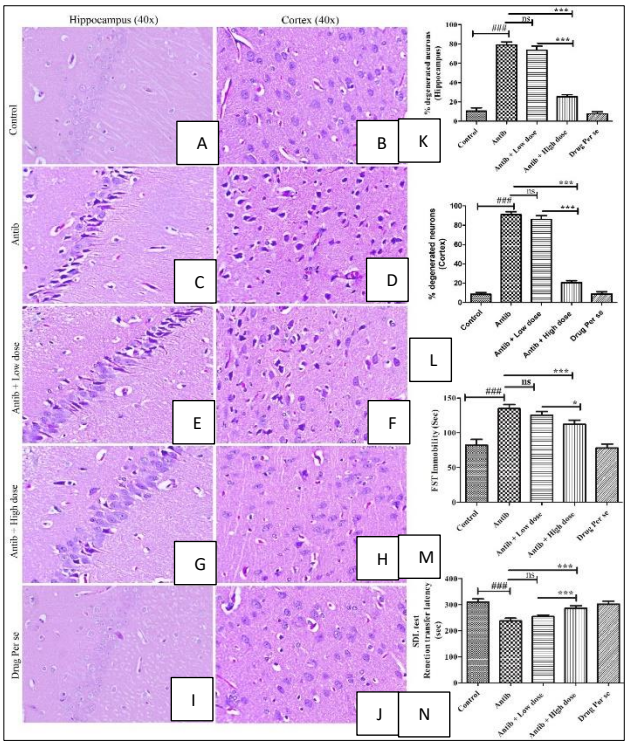

Figure 9 (A-N): Histopathological damage in the brain tissue and compromised neurobehavioral attributes in the Antib-treated group. The low dose of C-PRO failed to exhibit reversal of structural damage and improvement in neurobehavioral attributes, whereas the high dose of C-PRO effectively reversed the structural damage and biochemical parameters towards normal. H&E; 50 µm; 400x.

DISCUSSION

Antibiotic-induced gut dysbiosis and associated complications are major healthcare challengesworldwide.[2] In recent times, increased off-label use of various antibiotics has put a large population at risk of resistance. Moreover, the short-term and long-term consequences of various antibiotics are being explored, and attempts have been made to minimize their side effects. For instance, regular consumption of ampicillin at therapeutic dose induced microbial dysbiosis in the colon tissues of a mouse model, leading to enhanced production of NF-κB.[20] Antibiotic cocktails comprising vancomycin, neomycin, ampicillin, and metronidazole can hugely affect the microbiota diversity in the gut and, on the other hand, decline the intensity of autoimmune uveitis by lessening effector T cells and cytokines in the gut and extraintestinal tissues.[21] Similar complications have been reported with various antibiotic cocktails in preclinical studies.[1,2,5,6,10] The gut microbiota and related metabolites play an important role in the host's immune response. Still, very little knowledge is available regarding microbiota interactions, their bi-products, and related inflammatory responses in the gut and other vital organs such as the heart, liver, kidney, lungs, spleen, testis, and brain. Therefore, in the present study, we tried to explore the same lapses with the help of animal studies and treating them with an antibiotic cocktail. We further explored the protective potency of C-PROs in the gut and other vital organs using biochemical and histopathological analysis. Herein, we have used the combination of coconut oil and probiotics since both these products have marked antioxidant, anti-inflammatory, gut protective potency, and also protective effects for compromised vital functions in the preclinical studies. Hence, we here tried to explore the synergistic protective effect of coconut oil and probiotics against compromised vital organ functioning and derailed biochemical parameters caused by an antibiotic cocktail.

In the present study, we analyzed the change in body weight of different treatment groups weekly for 4 weeks. We found that the treated group had declined BW from week 1 onwards, and the reason for this could be a damaged gastrointestinal tract, compromised gut microbiota, and associated reduced food intake. The low dose of C-PRO failed to reverse the reduced BW towards normal, whereas the high dose of C-PRO showed an increase in BW from week 1 onwards as compared to the Antib-treated groups. We inferred that the gut protective effect of the C-PRO effect might be accountable for increased BW (Figure 1). Also, the protective effect of C-PRO was seen from day seven onward.

The oxidative stress caused by antibiotics is well established. These antibiotics, on the one hand, reduce the antioxidant activity of SOD, and CAT and reduce the level of GSH; on the other hand, increase the level of TBARS via lipid peroxidation, which eventually together manifests into oxidative stress.[22] The imposed oxidative stress further causes gastrointestinal toxicity and also affects the vital organs such brain, heart, liver, kidney, spleen, lungs, and testis, along with altering various biochemical parameters.[2,23-27] Considering these facts, in the present study, we have estimated the marker of oxidative stress. We found that the antibody-treated group exhibited marked oxidative stress, and a high dose of C-PRO effectively reversed the derailed markers of oxidative stress toward normal (Figure 2). We inferred that the synergistic antioxidant effect of coconut oil and probiotics might be accountable for this marked antioxidant effect of C-PRO. One of the primary concerns with the use of antibiotics is their gastrointestinal toxicity. Considering these facts, we studied the effect of Antib for the effect against structural damage in the stomach, duodenum, and colon, along with the length of the colon. We found marked damage to the stomach, duodenum, and colon in the anti-treated groups, along with the reduced colonic length, and the findings are aligned with various other published reports. The low dose of C-PRO, when tested for these compromised structural damages and reduced colon length, showed no marked improvement. The high dose of C-PROs showed a significant reversal of histopathological damage towards normal, and we inferred that the synergistic antioxidant effect of coconut oil and probiotics

might be accountable for this protective effect (Figures 3 and 4).[3,5,10]

To further explore the extent of Antib on various vital organs, we also studied the changes in histopathological damage to the lungs, spleen, and testis. We found that Antib caused marked damage to seminiferous tubules, Leydig cells, and spermatogonia, along with cellular disintegration. Similarly, marked damage was seen in the spleen, where the red pulp, white pulp, and marginal zone were indistinguishable. The lung tissue also showed macrophagic infiltration, congestion, and a thickened alveolar wall. The low dose of C-PRO, when tested for these compromised structural damages, no marked improvement was found but the high dose of C-PROs showed a significant reversal of histopathological damage towards normal (Figure 5).

To further strengthen our findings, we explored the deleterious effect of Antib on the histopathology of the heart, liver, and kidney, along with their specific injury markers. Although we could not find any direct impact of antibiotics on cardiovascular tissue, but gut-heart axis is well studied, and any damage to the gut axis will eventually reflect compromised cardiovascular functions.[28] It is well known that the gut and liver interact with each other via blood circulation, the portal vein, and the biliary tract, with nearly 70% of the blood supply in the liver coming from the portal vein.[29] The liver is the prime organ to get gut microbial products and by-products when intestinal venous blood is pumped out into the portal vein. This connection between the gut microbiota and the liver is referred to as an egg and hen.[30] Hence, it becomes necessary to understand how oral antibiotics may affect this sensitive balance and bring about possible lasting impacts on gene expression and the general health of the host. The major influences of gut microbiota and liver tissue diseases have been reported. One such example was demonstrated in patients with alcoholic liver disease, where a significant reduction in the C-PRO bacterial load, such as *Lactobacillus spp.*,*Bifidobacterium* spp., and *Enterococcus faecalis*, was found.[31,32] Additional research confirms a link involving the gut microbiota and non-alcoholic fatty liver ailments like cirrhosis and hepatocellular carcinoma.[33-36] Apart from the impact of antibiotics on the liver, their use is directly associated with compromised renal function, and various studies have shown the deleterious effect of antibiotics on compromised renal functions, both structurally and biochemically.[37]

We herein found that the Antib-treated group showed significant structural damage to the heart, liver, and kidney along with increased levels of LDH, CK-KM, ALT, AST, GGT, albumin, creatinine, urea, uric acid, and BUN, which are as per the previously published reports. Primarily, we found marked cellular disintegration, myofibril widening, pyknosis in cardiac tissue; pyknosis, congestion in the central vein, fibrotic changes, and cellular disintegration in liver tissue; damaged PCT, DCT, Bowmans's capsules, and basement membrane in the kidney when treated with an antibiotic cocktail. The low dose of C-PROs, when tested for these compromised structural damages and derailed biochemical parameters, no marked improvement was found, but the high dose of C-PROs showed a significant reversal of histopathological damage and biochemical parameters towards normal (Figures 6-8).

Antibiotic-induced neurotoxic and behavioral manifestations are one of the major concerns. Numerous studies have reported the deleterious effect of antibiotics on the CNS.[5,9] The use of antibiotics has been reported to alter the release of various neurotransmitters, causing cognitive decline and depressive-like behavior.[38] The association of the gut-brain axis has been explicitly studied and is well-established now. Numerous proofs are available to back that gut microbiota affects the action and metabolism of tryptophan-derived intermediaries such as serotonin and kynurenine.[38-41] It is shown in the Antib-induced stressed mice their novel object recognition memory is improved by obstruction of serotonin reuptake by fluoxetine.[32,42] Furthermore, the accumulation of corticosterone in plasma was increased in antibiotic-treated mice, and their increased circulation might affect the cognitive deficit as presented in antib-treated mice.[42-44] Furthermore, the increased plasma corticosterone in antibiotic-treated mice also causes downregulation of BDNF, leading to compromised cognitive function.[45,46] Moreover, the disrupted gut-brain axis also causes neurotoxicity and neurobehavioral dysfunction because of neuroinflammation or oxidative stress, as seen in various neurodegenerative disorders. Specifically, in Alzheimer's disease, disrupted gut-brain axis was found to be associated with depressive behavior and declined cognitive function.[47] Considering these facts, we herein explored the effect of Antib in terms of histological alterations in the hippocampus and cortex, along with the effect on depression and cognitive function, using the FST and SDL tests. We found that in the antib-treated group, the percentage of degenerated neurons was significantly higher as compared to the control group. Also, in the Antib-treated group, the duration of immobility for FST was increased, which signifies depressive-like behavior. Additionally, in the Antib-treated group, the duration of retention transfer latency was significantly reduced when compared to the control group, and hence, signifies reduced cognitive function. The low dose of C-PRO, when tested for these compromised structural damages and neurobehavioral attributes, no marked improvement was found, but the high dose of C-PRO showed a significant reversal of histopathological damage, reduced depressive-like behavior, and reduced declined cognitive functions and biochemical parameters towards normal (Figure 9).

CONCLUSION

The present study showed the deleterious effect of Antib in a mouse model, which was reflected in terms of reduced body weight, marked oxidative stress, and histopathological damage to the stomach, duodenum,

colon, brain, heart, liver, lungs, kidney, spleen, and testes. The exposure to Antib also caused alteration in biochemical parameters such as markers of cardiac injury (LDH and CK-MB), liver injury (ALT, AST, GGT, and albumin), markers of renal injury (creatinine, urea, uric acid, and BUN). Additionally, we found that antibody exposure in mice exhibited depressive-like behavior and declined cognitive function. The treatment with a low dose of C-PROs showed negligible protective effect, whereas a high dose of antibiotics effectively reversed the structural, biochemical, and neurobehavioral attributes toward normal. We conclude that the synergistic multifactorial effect of coconut oil and probiotics might be responsible for multidimensional protective effects against compromised gastrointestinal attributes and associated complications. However, more detailed cellular and molecular-based studies are needed to substantially strengthen the findings.

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